

The University of Chicago

HISTOLOGICAL STUDIES ON THE
ROOT OF MELILOTUS ALBA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1941

By

FRANCES RANNEY BOTTUM

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Reprinted from
THE BOTANICAL GAZETTE
Vol. 103, No. 1, September 1941

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HISTOLOGICAL STUDIES ON THE ROOT OF *MELILOTUS ALBA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 527

FRANCES R. BOTTUM

(WITH FOURTEEN FIGURES)

Introduction

The distribution, soil adaptation, habits, and agricultural value of biennial white sweet clover, *Melilotus alba* Desv., have been extensively investigated, a notable survey being that of LLOYD (7). Studies of the relative growth rates of roots and tops have been made by SNIDER and HEIN (11), WILLARD (15), and MARTIN (9). MARTIN has also described the germination and establishment of the seedling. The habit of growth of the root system has been investigated by WEAVER (14). COOPER (1), GUIGNARD (4), WATT (13), and YOUNG (20) have given accounts of the embryogeny; and the cytology of the meristematic cells of the root tip has been investigated by ELDERS (2). MCMURRY and FISK (10) have reviewed those points in earlier studies of seedling structure in the Leguminosae by DE CANDOLLE, VAN TIEGHEM, NÄGELI, GERARD, and COMPTON which are of significance in the study of *Melilotus*, and they have described the transition of root to stem structure in the seedling.

The object of this investigation is to supplement these studies with a description of further details of the root structure, especially of the older roots.

Material and methods

The plants used in this study were grown in the field, except very young seedlings for which pots of soil or germination dishes were used. The seed coats were softened by treatment with concentrated sulphuric acid, employing the method of LOVE and LEIGHTY (8), except that the time was shortened from 15 to less than 5 minutes.

Inoculation of soil with nodule-forming bacteria was insured by mixing the seeds, before sown, with fresh soil taken from around the roots of healthy older plants growing elsewhere. Plants were collected at convenient intervals throughout the period of growth.

Whole seedlings and short pieces of older roots were killed and fixed in formalin-acetic-alcohol or in Navashin's solution. The methods of clearing seedlings and free-hand sections in chloral hydrate or in cedar oil, of maceration, and of imbed-

ding in paraffin were used. Xylol was the clearing agent employed, and sections of roots were made at 8 or 12 μ . They were stained with Flemming's triple stain or with safranin and fast green.

Observations

PRIMARY ROOT OF SEEDLING

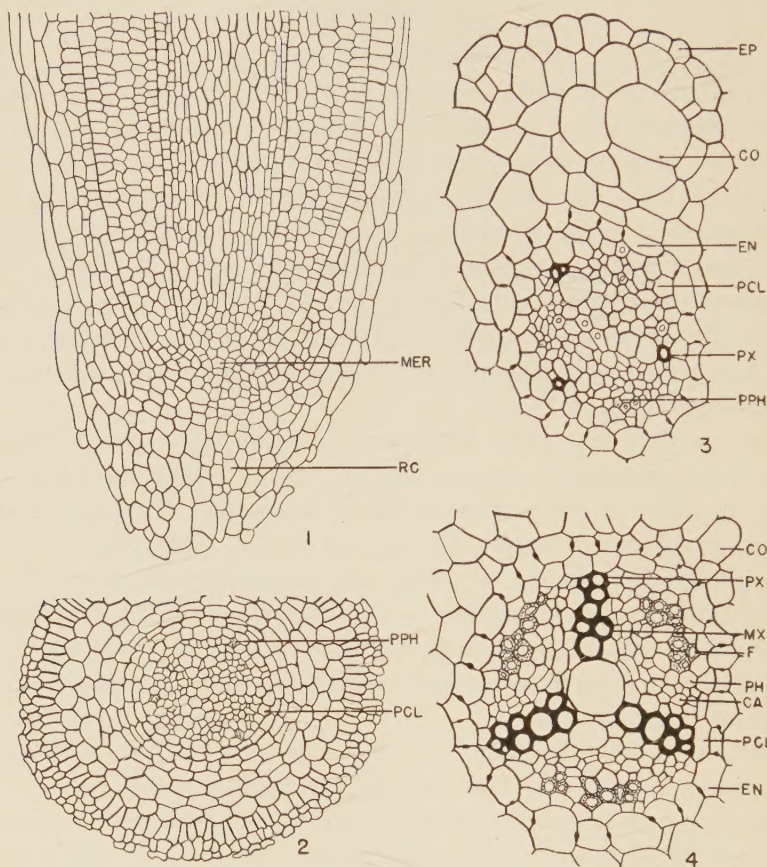
In the embryo of the seed, according to MARTIN (9), the radicle, hypocotyl, cotyledons, plumule, and even the crown buds are recognizable. In germination of the seed, the primary root on emerging penetrates the soil and forms a slender unbranched tap root. The ontogeny of the primary root corresponds to the fourth angiospermous type as described by JANCZEWSKI (5). Growth is accomplished by the activity of a transverse plate of meristem from which the stele, cortex, and central cone of tissue of the root cap develop. A lateral meristem, continuous near the root tip with the terminal meristem, gives rise by radial and periclinal divisions of the cells to the outer portion of the calyptra, and proximally constitutes a distinct dermatogen (fig. 1).

The root cap is composed of closely packed cells with conspicuous nuclei and relatively dense cytoplasm, except in the outer layers of cells which are separating from the cap. The young root immediately proximal to the terminal meristem shows a central region of small cells, varying in size, surrounded by one or two sheaths of larger cells, many of which are undergoing conspicuous tangential division and are densely cytoplasmic. Outside this central core is a peripheral region of large cells with deeply staining nuclei and dense cytoplasm, and the sheath of dermatogen with a few loosened layers of root-cap cells. There are no clearly defined plerome and periblem.

The first discernible differentiation of stelar tissue is the very early appearance of three protophloem strands abutting a well-defined uniseriate pericycle (fig. 2). These alternate with ridges of a triradiate central mass of larger parenchymatous cells, many of which later constitute the primary xylem. The cells of the protophloem are conspicuous because of their radial arrangement, the thinness of their cytoplasm, and the fact that their lateral walls are compressed by the adjacent meristematic cells. In longitudinal sections they appear to have elongated while adjacent cells continued to divide. The end walls are not tapering and there are no sieve plates. They retain their identity until after the protoxylem is differentiated. At the level of first appearance of the protophloem, the outermost layers of cortical cells become spherical, and intercellular spaces appear. The epidermis becomes clearly defined by the smaller size, long radial axis, and compactness of its cells.

In the region of most rapid elongation the metaphloem is differentiated, the sieve tubes developing conspicuous slime plugs very early. Companion cells ap-

pear at the same time but fibers are differentiated slightly later. Root hairs are not yet formed in this region but first appear where differentiation is most active. They are not confined to a definite zone but persist on all roots until the epidermis



FIGS. 1-4.*—Primary root of seedling: Fig. 1, longisection of root tip. Fig. 2, transection showing differentiation of protophloem and pericycle. Fig. 3, same at level of differentiation of protoxylem and endodermis. Fig. 4, same at level of completion of primary differentiation and formation of cambium.

* Symbols in all figures: *b*, tissue infected with bacteria; *ca*, cambium; *co*, cortex; *en*, endodermis; *ep*, epidermis; *f*, fiber; *mer*, meristem; *mx*, metaxylem; *pcl*, pericycle; *pd*, periderm; *ph*, phloem; *pi*, pith; *pph*, protophloem; *px*, protoxylem; *r*, ray; *rc*, root cap; *trc*, temporary root cap; *v*, vascular strand; *xy2*, secondary xylem.

is lost. Near the level of formation of the youngest root hairs the endodermis and protoxylem ridges are differentiated (fig. 3). The former consists of a single layer of the innermost cortical cells adjacent to the pericycle and having narrow Casparian bands. Each protoxylem ridge consists of one to four adjacent spiral or annular vessels, the outermost being smaller and next to the pericycle. The primary

root is triarch most commonly, but is not infrequently tetrarch, as shown by McMURRY and FISK (10) in their figures illustrating the condition of the tap root at the close of primary differentiation.

Whereas the outermost phloem cells differentiate as fibers first, sometimes giving the appearance of six bundles of fibers, others may soon form between them from parenchymatous cells adjacent to the pericycle, so that the whole phloem mass is frequently capped by fibers. Parenchyma, usually one or two layers of cells, separates the phloem and xylem. In this the cambium later arises by divisions tangential to the metaxylem (fig. 4).

Lignification of the metaxylem elements, which consist of cells with reticulate or simple-pitted walls, proceeds centripetally to include most of the central cells of the stele, though frequently some of these remain parenchymatous. Occurrence of an unusually large central metaxylem vessel is not uncommon.

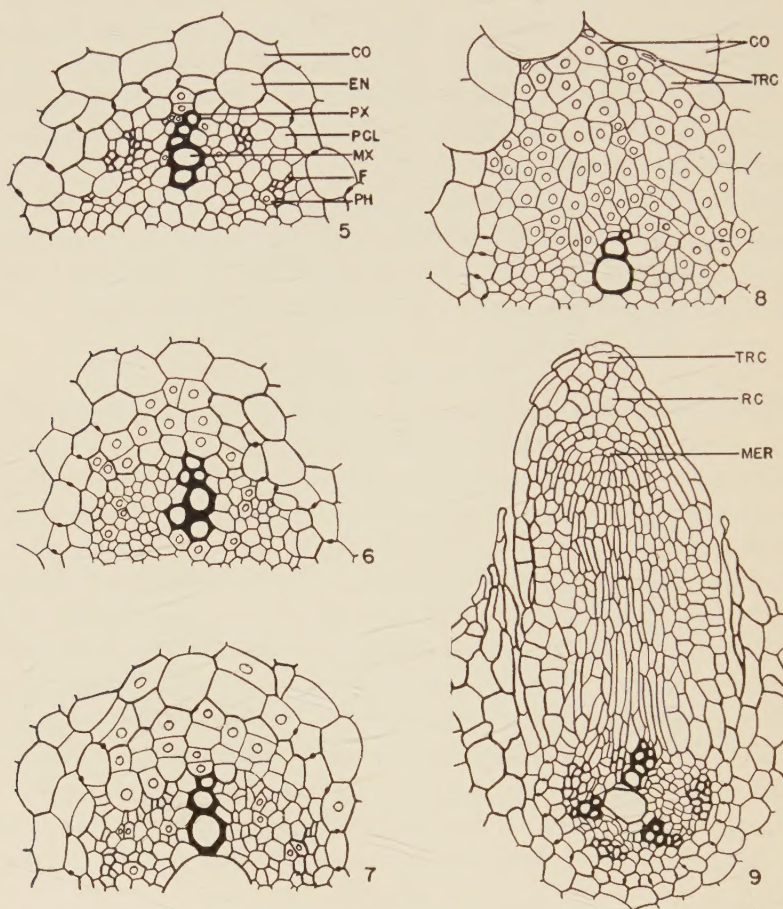
SECONDARY ROOTS

Before initiation of secondary growth and near the close of primary differentiation in the young tap root, lateral roots develop. These start growth through the periclinal divisions of a single pericyclic cell next to a protoxylem vessel, followed by similar divisions of a plate of adjacent pericyclic cells. Two successive periclinal divisions occur, followed by radial divisions and by stretching and enlargement of the overlying endodermal cells (figs. 5-7). As growth by further activity of pericyclic cells proceeds, the endodermal cells next to this new tissue likewise divide radially and tangentially, forming a temporary root cap at the tip, two or three cells in thickness. Most of the temporary cap cells are crushed against the cortical cells of the older root, are digested, or—after emergence of the root tip—are worn off or crushed by the soil. The cortical cells in turn are crushed, digested, or torn and pushed aside mechanically by the emerging root (figs. 8, 9).

The secondary roots develop a vascular system in a manner similar to that of the primary root. They are triarch usually, sometimes diarch, with a conspicuous endodermis.

During the third and fourth months, by which time a large amount of secondary tissue has developed near the crown of the plant, secondary roots arise in abundance on older roots and on the hypocotyl. Although the first node above the hypocotyl and the bud in the axil of the unifoliate leaf may become buried by soil and litter and remain alive during the winter of the first year, no adventitious roots were observed to arise above the first node. Adventitious and secondary roots, arising late in ontogeny, appear always opposite the primary rays, singly or in groups of two or three, emerging often close to the stump of an earlier rootlet which has died. The emergence of such roots is especially conspicuous during late autumn and winter months. Their formation begins in very early summer, when

the diameter of the primary root scarcely exceeds 1 mm., so that when the crown buds of the second year begin active growth, these roots have themselves branched and bear root nodules.



FIGS. 5-9.—Development of secondary root: Fig. 5, transection of portion of primary root showing initiation of secondary root by division of single pericyclic cell. Fig. 6, same showing division tangentially of plate of pericyclic cells and radial division of endodermal cell. Fig. 7, same showing second tangential division of pericyclic cells and tangential division of endodermal cells. Fig. 8, longisecision of young branch root showing crushing and digestion of cells of cortex of primary root and of temporary root cap. Fig. 9, transection of primary root showing emergence of secondary root.

The adventitious roots arise by meristematic activity of the cork cambium and underlying pericyclic cells. The first vascular tissue of the young root differentiates through derivatives of the ray to that of the older root. The outer and more distal parts of the adventitious root are derived from the periderm, and its vascu-

lar system is later continued by differentiation of cells of its own histogen. As the adventitious root emerges, its base is surrounded by a glistening white mass of cells, formed by the cork cambium, which continues as a thin sheath over the root tip. The sheath is finally crushed, its cells disintegrating and leaving the young root free.

Subsequent development is not unlike that of other roots. The endodermis is more conspicuous than it is in the tap root. The adventitious roots are diarch or triarch most commonly, though tetrarch—and rarely pentarch—roots were found.

SECONDARY STRUCTURES

Cambial activity in the primary root begins when the seedling is 7–8 days old (10). During the third and fourth weeks of growth of the seedling and before the first trifoliate leaf has appeared, the cambium, at first limited to cells between the metaxylem and phloem, comes to involve the cells of the pericycle which are adjacent to the protoxylem. This starts the development of the primary rays and completes the cambium cylinder as secondary growth proceeds. At this stage numerous secondary roots have appeared and attained lengths of 1–2 mm., root nodules have developed, and the tap root is about 3 inches long. Root hairs cover the entire root system except the tips distal to the region of differentiation. The cork cambium has arisen in the endodermis.

During the second month several trifoliate leaves appear, and the hypocotyl begins to withdraw by contraction underground. The tap root attains a length usually exceeding 1 foot, and rootlets of the third order have appeared. Root hairs persist except in the upper part of the tap root where they have been lost with the rest of the epidermis after differentiation of the periderm.

By the end of the fourth month the tap root may have become several feet in length. Two or three of the lateral roots have developed into large branches, nearly equaling the tap root in diameter at the level of their divergence and in depth reached in the soil. Most of the lateral roots, however, remain short. At this time the largest diameter of the tap root about equals that of the hypocotyl and stem.

Throughout the late spring and summer of the first season a large body of vascular tissue develops, parenchyma remaining relatively inconspicuous. Transverse sections of the tap root show a compact mass of primary and secondary xylem and numerous narrow rays. The primary phloem persists, as yet uncrushed by secondary growth. The periderm is about twelve cells in thickness, and its outer suberized cells have sufficiently thick walls to show pits distinctly.

Cells of the vascular cambium are arranged in tiers as shown in longitudinal sections. They taper abruptly at the ends and are about $150\ \mu$ in length, those of the rays being more nearly isodiametric. The zone of meristem between the secondary xylem and phloem is three to five cells wide. Like the cambial cells, the sieve tubes

and companion cells of the secondary phloem are arranged in tiers, while gliding growth is characteristic of cells differentiating as fibers.

Fibers in the root are confined to the phloem and secondary xylem, not occurring in the primary xylem, cortex, pericycle, or periderm. Those in the xylem often show distinct pits. They vary greatly in length, those measured being 0.6–3 mm. in length at maturity.

The earliest formed secondary xylem consists of a few vessels with long slender pits and very large diameter, scattered within compact tissue composed mostly of fibers or small tracheids, and a few parenchymatous cells grouped mainly around the vessels. Many of the parenchymatous cells later develop thick pitted walls distinguishing the earlier secondary growth from that occurring later.

The portion developed after the second month has somewhat less compact xylem (figs. 10, 11). Cells of the cambium differentiate as new rays; the primary rays widen until they are, by the fourth month, six or more cells in thickness near the cambium. There is a tendency for cambium initials to differentiate as fibers for some time, and then for a period to differentiate as vessels and tracheids in close succession, so that alternating layers of fibers and conducting tissue resemble rings of growth. No striking contrast, however, distinguishes the growth of the first season from that of the second in most of the root. This is not true in the hypocotyl and uppermost part of the tap root. In these, during the winter months, more cambium cells remain parenchymatous and the vessels formed are small. In winter and early spring of the second season large vessels and numerous tracheids are again formed, giving a contrast which serves, for a while at least, to demark the two seasons of the vegetative cycle. JONES (6), working with alfalfa, reports layers of crushed phloem in addition, indicating successive years' growth. This was not seen in *Melilotus*.

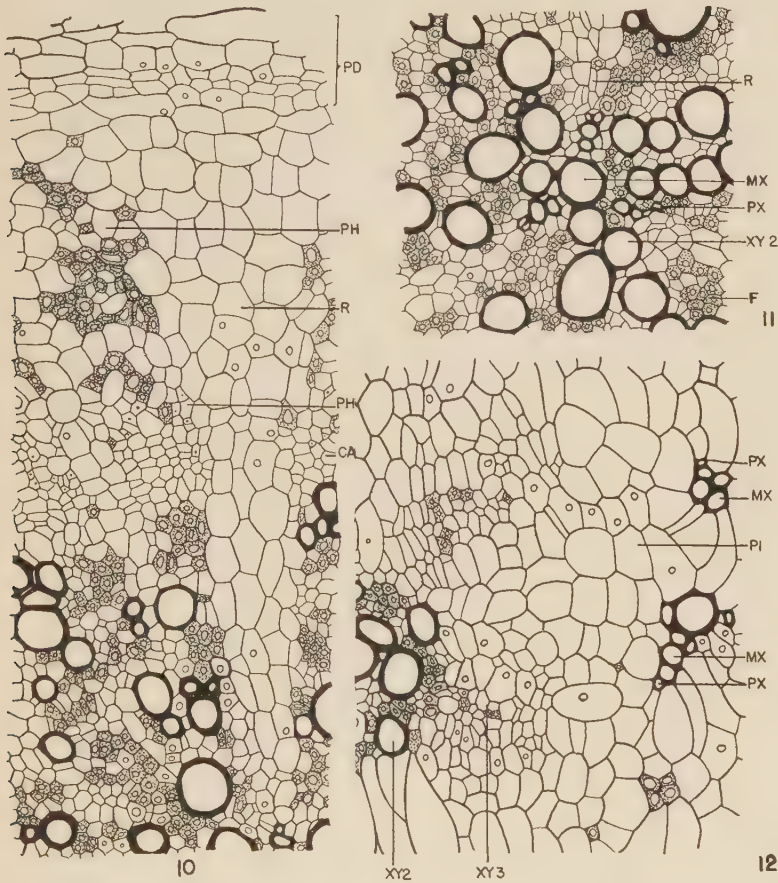
During the autumn and first season the most striking feature of secondary growth is the development of storage tissue. In this connection it will be necessary to consider the hypocotyl.

HYPOCOTYL AND DEVELOPMENT OF STORAGE TISSUE

In their study of the transition region in *Melilotus alba*, MCMURRY and FISK (10) describe the change from the typical triarch condition of the primary root to diarchy or tetrarchy in the lower hypocotyl and the disappearance of the exarch ridge of xylem on the intercotyledonary axis at higher levels, so that there is no direct connection with the primary xylem of cotyledonary or plumular traces. They mention also the differentiation of a conspicuous pith in the hypocotyl of the seedling.

Secondary growth from a vascular cambium proceeds in the hypocotyl in a manner similar to that in the root. Likewise a cork cambium develops in the endo-

dermis continuous with that of the upper part of the tap root, and at the same time. It forms, however, a conspicuous layer of secondary tissue before loss of the primary cortex and epidermis, which occurs after the hypocotyl is completely underground. The hypocotyl is thenceforth not easily distinguishable externally

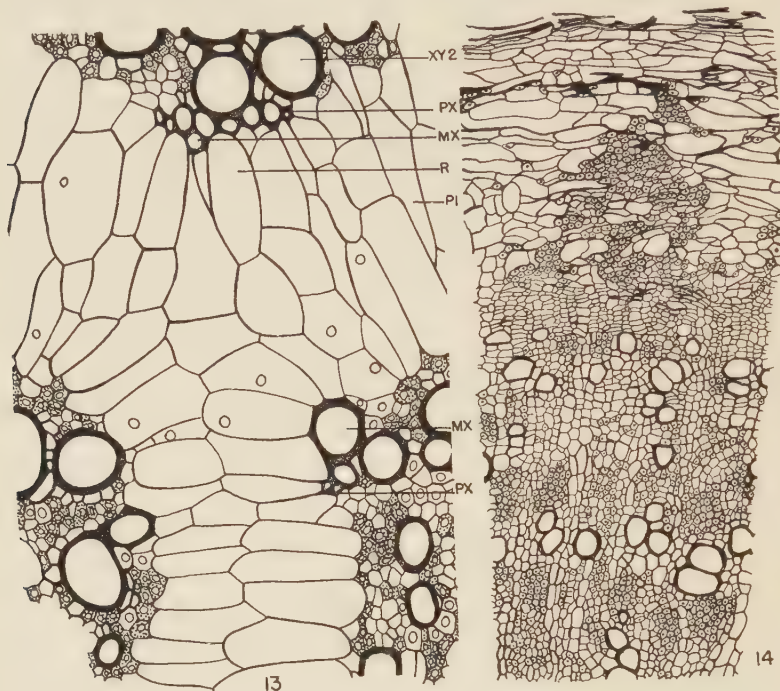


FIGS. 10-12.—Tap root of older plant: Fig. 10, transection of portion of tap root in June of first year showing secondary tissues. Fig. 11, same showing primary and secondary structures of central portion of stele. Fig. 12, central portion of tap root of second year showing tertiary tissue and separation of primary xylem elements by growth of parenchyma cells.

from the tap root. Internally the arrangement of the primary xylem makes such distinction possible until rapid growth of storage tissue involves the central elements of the stele.

In the tap root and hypocotyl, enlargement and radial divisions of cells in the pericycle and between the phloem groups become conspicuous in about the fourth

month, followed during the summer by general proliferation of the parenchyma, there and in the phloem itself. Phloem fibers become separated singly or in groups, and follow a sinuous course. During the same months all the rays are widened by multiplication and enlargement of their cells, but especially the central portions of the primary rays. Parenchyma associated with the metaxylem becomes active, the cells enlarge greatly, elongate tangentially, divide transversely, and then enlarge again. This results in separation of the metaxylem elements and in crushing



FIGS. 13, 14.—Tap root of plants of second year: Fig. 13, transection of central portion showing separation of elements of primary wood by growth of parenchyma. Fig. 14, transection of portion of dead root showing crushed phloem and thickened walls of parenchyma cells throughout most of area.

of the protoxylem (figs. 12, 13). The hypocotyl, upper part of the tap root, and larger secondary roots thus appear to possess a pith. Parenchymatous cells laid down close to the vessels of the secondary xylem, hitherto inconspicuous, enlarge and force the vessels and fiber groups apart. The activity of these cells in the central region of the hypocotyl is especially striking. The accumulation of starch in the storage tissue during the first season and its decrease during the second season have been described by WILLARD (15). Increase of the storage tissue continues in the second season but at a slower rate. A small amount of tertiary thickening may

occur in the pith, the cells maturing as fibers when any differentiation occurs (fig. 13).

What is apparently pith, which develops in the hypocotyl by proliferation of ray and xylem parenchyma, is to be distinguished in origin from the pith which first appears in the seedling associated with the primary tissues above the divergence of the two cotyledonary traces. In the old hypocotyl that pith appearing in the seedling is still conspicuous in the region of the cotyledonary node and is continuous with the pith of the stem rather than with that of the lower part of the hypocotyl, from which it is separated by a transverse plate of lignified tissue in which the parenchyma at the center of the stele becomes thick walled. This lignified tissue is confined to the cotyledonary node, suggesting that the hypocotyl in its structure is more nearly similar to the root than to the stem. It is readily distinguishable from the stem late in ontogeny as well as in the seedling.

Longitudinal sections through the old hypocotyl, at the time of flowering in the second season, show the primary vascular tissue and older vessels—and fibers of the secondary xylem in the central part of the stele—to be pushed laterally into sinuous folds by proliferating parenchyma, whereas the more recently formed vessels and fibers follow a straight course. This strongly suggests that proliferation of the parenchyma may have played an important role in the shortening and burial of the hypocotyl long before the cessation of secondary growth. JONES (6) suggests this explanation for shortening of the hypocotyl in alfalfa.

ROOT NODULE

The literature of the root nodule in the Leguminosae has recently been reviewed by FRED, BALDWIN, and MCCOY (3) and WILSON (16).

On the primary root of field-grown seedlings of *Melilotus* subjected only to chance infection by root nodule bacteria, nodules appear externally 7 days after germination of the seeds. At this age the unifoliate leaf has appeared but has not yet expanded. Even earlier than this nodules can be seen within the cortex of seedlings which have been cleared in chloral hydrate or in xylol. They become conspicuously enlarged and are more abundant by the tenth day, at which time the unifoliate leaf of the seedling is fully expanded. The fact that few nodules are formed before the appearance of the first true leaf in *Medicago* has been noted and regarded as significant by THORNTON (12). Secondary roots, after the appearance of root hairs, may also develop nodules.

Since divisions of the cells in the very young nodule are in many planes, a globular mass of tissue develops before the emergence of the nodule beyond the surface of the root. This enlarges and, after emergence, develops a cylindrical shape and becomes pink in color.

Transverse sections of mature nodules show the usual central mass of paren-

chymatous cells, most of which contain bacteria. A sheath of cells, containing starch in abundance, envelopes the central mass except at its distal end where a terminal meristem continually forms additional nodular tissue. The mature nodule has an outer cortical region of uninfected cells, beneath which—and outside the starch sheath—the nodule is traversed by two vascular strands differentiated back to the stele of the root opposite one, or occasionally two, of the protoxylem ridges.

The nodules of *Melilotus alba* are annual. Before the end of their growth they may become branched, the two vascular strands within the nodule likewise being branched repeatedly. At the end of the season the most central tissue of the nodule, containing the bacteria, disintegrate, leaving for a while a soft brown shell of cortical and vascular tissues. Eventually this too is lost and the bacteria within are returned to the soil.

A description of the ontogeny of the nodule is not undertaken here. Recent investigations of the possible role of growth substances, of metabolic products of the bacteria, and of growth-stimulating materials of the soil in the infection of roots and in the initiation and development of root nodules in the Leguminosae, and speculations in regard to these, have raised new questions and reopened old ones. Many of these have been pointed out by WILSON (16). Likewise cytological studies in which the tetraploid nature of the infected cells of the nodule has been described (17, 18, 19) seem to indicate that the whole matter of the ontogeny of the root nodule merits reinvestigation.

DEGENERATION AND DEATH OF ROOT

The period of flowering extends for about 6 weeks early in the summer of the second season. During this time the growth of the tops greatly exceeds that of the roots, and the weight of the root is reduced by loss of reserve foods (15). After subsequent formation of the last seeds the plant dies, usually in August or September. The root dies first.

Before death of the root, signs of degeneration appear. The older phloem is crushed by secondary growth, causing collapse of the parenchyma, sieve tubes, and some of the fibers. At the same time the interfascicular parenchyma collapses so that a sheath of dead tissue is formed beneath the periderm (fig. 14). Activity of the cambium ceases, as shown by the maturity of all the tissues most recently formed from it, so that the zone of undifferentiated cells between the xylem and phloem is narrowed down to a single layer of cells constituting the cambium. A marked increase in the number of fibers differentiated in the central part of the stele and general thickening of the walls of parenchymatous cells occur throughout the root, with the exception of the isolated periderm. Slight thickening of the walls of the youngest phloem and of the cambium cells takes place, occurring first in the rays, beginning near the center of the root, but becoming most marked in

the second year's growth. These changes occur first in the younger roots, progress to the older ones, and finally involve the tap root and hypocotyl.

The earliest indication of death of the tissues is shriveling of the cytoplasm. In sections of larger roots made before they seem to be dead, brown or black discolored areas occur. These mark the bases of dead lateral roots whose phloem gives the staining reactions characteristic of necrotic tissue. Through these lateral roots saprophytic fungi soon invade the larger roots, attacking the phloem, and then—following the rays inward—destroy the pith and finally plug the vessels by their growth. The entire life of the biennial plant occupies about 19 months from the time of germination of the seed.

Summary

1. The ontogeny of the primary root of *Melilotus alba* is of the fourth angiospermous type of JANCZEWSKI, in which there is a general meristem from which arises the cortex, stele, and central portion of the root cap. The meristem is extended laterally to form the outer parts of the root cap, and at higher levels a dermatogen.

2. Protophloem is the earliest stelar tissue differentiated.

3. Secondary roots arise by cell divisions in the pericycle opposite the protoxylem ridges. The endodermis forms a temporary root cap, which is ultimately crushed or digested, as are also cells of the cortex and epidermis of the primary root by the emerging root. The vascular system is developed in a manner similar to the primary root.

4. Lateral roots develop in the periderm and primary rays of old roots and of the hypocotyl. Cells of the periderm, by radial and tangential divisions, give rise to a temporary sheath over the young root, which is later ruptured. Subsequent development is similar to that of the secondary roots.

5. Secondary tissues develop from a cork cambium which arises in the endodermis, and from a vascular cambium which arises in the seedling between the xylem and phloem. A small amount of tertiary tissue may develop in the pithlike mass of the stele of the tap root late in ontogeny, maturing chiefly as fibers.

6. Parenchyma throughout most of the older roots, especially in the primary rays, becomes active late in the season, separating by its proliferation groups of vessels, fibers, and sieve tubes, crushing the protoxylem, and forming a pithlike mass. It serves as storage tissue for a large reserve of starch.

7. Withdrawal of the hypocotyl underground accompanies proliferation of the parenchymatous tissue and increase in the diameter of the hypocotyl and tap root late in the first season.

8. The hypocotyl has a pith above the level of divergence of cotyledonary traces continuous with that of the stem and present in the seedling. Late in on-

togeny it develops also a pithlike mass by proliferation of parenchyma in central parts of the stele, which is continuous with that of the root and homologous with it.

9. The root nodule originates in young roots near the close of primary differentiation. Two vascular strands are differentiated which are connected with the stele of the root opposite the nearest protoxylem ridges. An apical meristem adds cells to a central mass of infected and starch-bearing tissues and to a cortex which invests the entire nodule.

10. Root nodules are annual. New ones develop in succession on young roots.

11. Secondary growth continues in the second year through the period of flowering, but at a slower rate. Degeneration occurs after seeds are formed. The older phloem is crushed, the periderm becomes isolated by a layer of dead tissue beneath, most parenchymatous tissue throughout the root develops thick walls, and the cambium ceases to form new tissue. Increase of fibers in the center of the stele is marked.

12. The roots die before the tops; small roots die before the large ones. The tissues finally become disintegrated by the action of saprophytic fungi. Nineteen months is the length of life of the plant from the time of germination of the seed.

GEORGE PEABODY COLLEGE FOR TEACHERS
NASHVILLE, TENNESSEE

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